

WHAT IS CLAIMED IS:

- 1 1. An isolated protein comprising a HER-2/neu extracellular domain
2 fused to a HER-2/neu phosphorylation domain, wherein the protein is capable of
3 producing an immune response in a warm-blooded animal.
- 1 2. The protein of claim 1, wherein the protein has a sequence at least
2 80% identical to the sequence of SEQ ID NO:6, or wherein the protein comprises a
3 sequence at least 80% identical to the sequence of SEQ ID NO:3 fused to a sequence at
4 least 80% identical to the sequence of SEQ ID NO:4.
- 1 3. The protein of claim 1, wherein the protein comprises a sequence at
2 least 80 % identical to the sequence of SEQ ID NO:3 directly fused to an amino acid
3 sequence at least 80% identical to the sequence inclusive of Gln 991 to Val 1256 of SEQ
4 ID NO:2, or wherein the protein comprises a sequence at least 80 % identical to the
5 sequence of SEQ ID NO:3 fused to the amino acid sequence at least 80% identical to the
6 sequence inclusive of Gln 991 to Val 1256 of SEQ ID NO:2.
- 1 4. The protein of claim 1, wherein the protein comprises a sequence at
2 least 80% identical to the sequence of SEQ ID NO:8 directly fused to a sequence at least
3 80% identical to the sequence of SEQ ID NO:4, or wherein the protein comprises a
4 sequence at least 80% identical to the sequence of SEQ ID NO:8 fused to a sequence at
5 least 80% identical to the sequence of SEQ ID NO:4.
- 1 5. The protein of claim 1, wherein the protein comprises a sequence at
2 least 80% identical to the sequence of SEQ ID NO:8 directly fused to the amino acid
3 sequence inclusive of Gln 991 to Val 1256 of SEQ ID NO:2, or wherein the protein
4 comprises a sequence at least 80% identical to the sequence of SEQ ID NO:8 fused to a
5 sequence at least 80% identical to the amino acid sequence inclusive of Gln 991 to Val
6 1256 of SEQ ID NO:2.
- 1 6. The protein of claim 1, wherein the HER-2/neu extracellular
2 domain is fused to the HER-2/neu phosphorylation domain via a chemical linker.
- 1 7. The protein of claim 6, wherein the chemical linker is an amino
2 acid linker.

- 1 8. A nucleic acid molecule encoding the protein of claim 1.
- 1 9. A viral vector comprising a polynucleotide sequence encoding the
2 protein of claim 1.
- 1 10. A pharmaceutical composition comprising the protein of claim 1,
2 and a pharmaceutically acceptable carrier or diluent.
- 1 11. The pharmaceutical composition of claim 10, wherein the
2 pharmaceutical composition is a vaccine.
- 1 12. The pharmaceutical composition of claim 10, further comprising an
2 immunostimulatory substance.
- 1 13. The pharmaceutical composition of claim 12, wherein the protein is
2 presented in an oil-in-water emulsion.
- 1 14. The pharmaceutical composition of claim 12, wherein the
2 immunostimulatory substance is SBAS2, 3D-MPL, QS21, or a combination of 3D-MPL
3 and QS21.
- 1 15. A pharmaceutical composition comprising the nucleic acid
2 molecule of claim 8, and a pharmaceutically acceptable carrier or diluent.
- 1 16. The pharmaceutical composition of claim 15, wherein the
2 pharmaceutical composition is a vaccine.
- 1 17. The pharmaceutical composition of claim 15, further comprising an
2 immunostimulatory substance.
- 1 18. The pharmaceutical composition of claim 15, wherein the nucleic
2 acid molecule is a DNA molecule.
- 1 19. A method for eliciting or enhancing an immune response to HER-
2 neu protein, the method comprising the step of administering to a warm-blooded animal
3 the protein of claim 1 in an amount effective to elicit or enhance the immune response.

1 20. The method of claim 19, wherein the protein is administered in the
2 form of a vaccine.

1 21. A method for eliciting or enhancing an immune response to HER-
2 2/neu protein, the method comprising the step of administering to a warm-blooded animal
3 the nucleic acid molecule of claim 8 in an amount effective to elicit or enhance the
4 immune response.

1 22. The method of claim 21, wherein the nucleic acid molecule is in
2 the form of a vaccine.

1 23. The method of claim 21, wherein the step of administering
2 comprises transfecting cells of the warm-blooded animal *ex vivo* with the nucleic acid
3 molecule and subsequently delivering the transfected cells to the warm-blooded animal.

1 24. A method for eliciting or enhancing an immune response to HER-
2 2/neu protein, the method comprising the step of administering to a warm-blooded animal
3 the viral vector of claim 9 in an amount effective to elicit or enhance the immune
4 response.

1 25. The method of claim 24, wherein the step of administering
2 comprises infecting cells of the warm-blooded animal *ex vivo* with the viral vector and
3 subsequently delivering the infected cells to the warm-blooded animal.

1 26. An isolated protein comprising a HER-2/neu extracellular domain
2 fused to a fragment of the HER-2/neu phosphorylation domain, wherein the protein is
3 capable of producing an immune response in a warm-blooded animal.

1 27. The protein of claim 26, wherein the protein has a sequence at least
2 80% identical to the sequence of SEQ ID NO:7, or wherein the protein comprises a
3 sequence at least 80% identical to the sequence of SEQ ID NO:3 fused to a sequence at
4 least 80% identical to the sequence of SEQ ID NO:5.

1 28. The protein of claim 26, wherein the protein comprises a sequence
2 at least 80% identical to the sequence of SEQ ID NO:3 directly fused to a sequence at
3 least 80% identical to the amino acid sequence inclusive of Gln 991 to Arg 1049 of SEQ

4 ID NO:2, or wherein the protein comprises a sequence at least 80% identical to the
5 sequence of SEQ ID NO:3 fused to a sequence at least 80% identical to the amino acid
6 sequence inclusive of Gln 991 to Arg 1049 of SEQ ID NO:2.

1 29. The protein of claim 26, wherein the protein comprises a sequence
2 at least 80% identical to the sequence of SEQ ID NO:8 directly fused to a sequence at
3 least 80% identical to the sequence of SEQ ID NO:5, or wherein the protein comprises a
4 sequence at least 80% identical to the sequence of SEQ ID NO:8 fused to a sequence at
5 least 80% identical to the sequence of SEQ ID NO:5.

1 30. The protein of claim 26, wherein the protein comprises a sequence
2 at least 80% identical to the sequence of SEQ ID NO:8 directly fused to a sequence at
3 least 80% identical to the amino acid sequence inclusive of Gln 991 to Arg 1049 of SEQ
4 ID NO:2, or wherein the protein comprises a sequence at least 80% identical to the
5 sequence of SEQ ID NO:8 fused to a sequence at least 80% identical to the amino acid
6 sequence inclusive of Gln 991 to Arg 1049 of SEQ ID NO:2.

1 31. The protein of claim 26, wherein the HER-2/neu extracellular
2 domain is fused to the fragment of the HER-2/neu phosphorylation domain via a chemical
3 linker.

1 32. The protein of claim 31, wherein the chemical linker is an amino
2 acid linker.

1 33. A nucleic acid molecule encoding the protein of claim 26.

1 34. A viral vector comprising a polynucleotide sequence encoding the
2 protein of claim 26.

1 35. A pharmaceutical composition comprising the protein of claim 26,
2 and a pharmaceutically acceptable carrier or diluent.

1 36. The pharmaceutical composition of claim 35, wherein the
2 pharmaceutical composition is a vaccine.

1 37. The pharmaceutical composition of claim 35, further comprising an
2 immunostimulatory substance.

1 38. The pharmaceutical composition of claim 37, wherein the protein is
2 presented in an oil-in-water emulsion.

1 39. The pharmaceutical composition of claim 37, wherein the
2 immunostimulatory substance is SBAS2, 3D-MPL, QS21, or a combination of 3D-MPL
3 and QS21.

1 40. A pharmaceutical composition comprising the nucleic acid
2 molecule of claim 33, and a pharmaceutically acceptable carrier or diluent.

1 41. The pharmaceutical composition of claim 40, wherein the
2 pharmaceutical composition is a vaccine.

1 42. The pharmaceutical composition of claim 40, further comprising an
2 immunostimulatory substance.

1 43. The pharmaceutical composition of claim 40, wherein the nucleic
2 acid molecule is a DNA molecule.

1 44. A method for eliciting or enhancing an immune response to HER-
2 2/neu protein, the method comprising the step of administering to a warm-blooded animal
3 the protein of claim 26 in an amount effective to elicit or enhance the immune response.

1 45. The method of claim 44, wherein the protein is administered in the
2 form of a vaccine.

1 46. A method for eliciting or enhancing an immune response to HER-
2 2/neu protein, the method comprising the step of administering to a warm-blooded animal
3 the nucleic acid molecule of claim 33 in an amount effective to elicit or enhance the
4 immune response.

1 47. The method of claim 46, wherein the nucleic acid molecule is in
2 the form of a vaccine.

1 48. The method of claim 46, wherein the step of administering
2 comprises transfecting cells of the warm-blooded animal *ex vivo* with the nucleic acid
3 molecule and subsequently delivering the transfected cells to the warm-blooded animal.

1 49. A method for eliciting or enhancing an immune response to HER-
2 2/neu protein, the method comprising the step of administering to a warm-blooded animal
3 the viral vector of claim 34 in an amount effective to elicit or enhance the immune
4 response.

1 50. The method of claim 49, wherein the step of administering
2 comprises infecting cells of the warm-blooded animal *ex vivo* with the viral vector and
3 subsequently delivering the infected cells to the warm-blooded animal.

1 51. An isolated protein comprising a HER-2/neu extracellular domain
2 fused to a HER-2/neu intracellular domain, wherein the protein is capable of producing an
3 immune response in a warm-blooded animal.

1 52. The protein of claim 51, wherein the protein comprises a sequence
2 at least 80% identical to the sequence of SEQ ID NO:3 fused directly to a sequence at
3 least 80% identical to the amino acid sequence inclusive of Lys 676 to Val 1255 in SEQ
4 ID NO:1, or wherein the protein comprises a sequence at least 80% identical to the
5 sequence of SEQ ID NO:3 fused to a sequence at least 80% identical to the amino acid
6 sequence inclusive of Lys 676 to Val 1255 of SEQ ID NO:1 via at least one of a chemical
7 or amino acid linking group.

1 53. The protein of claim 51, wherein the protein comprises a sequence
2 at least 80% identical to the sequence of SEQ ID NO:3 directly fused to a sequence at
3 least 80% identical to the amino acid sequence inclusive of Lys 677 to Val 1256 of SEQ
4 ID NO:2, or wherein the protein comprises a sequence at least 80% identical to the
5 sequence of SEQ ID NO:3 fused to a sequence at least 80% identical to the amino acid
6 sequence inclusive of Lys 677 to Val 1256 of SEQ ID NO:2 via at least one of a chemical
7 or amino acid linking group.

1 54. The protein of claim 51, wherein the protein comprises a sequence
2 at least 80% identical to the sequence of SEQ ID NO:8 directly fused to a sequence at
3 least 80% identical to the amino acid sequence inclusive of Lys 676 to Val 1255 of SEQ
4 ID NO:1, or wherein the protein comprises a sequence at least 80% identical to the
5 sequence of SEQ ID NO:8 fused to a sequence at least 80% identical to the amino acid

6 sequence inclusive of Lys 676 to Val 1255 of SEQ ID NO:1 via at least one of a chemical
7 or amino acid linking group.

1 55. The protein of claim 51, wherein the protein comprises a sequence
2 at least 80% identical to the sequence of SEQ ID NO:8 directly fused to a sequence at
3 least 80% identical to the amino acid sequence inclusive of Lys 677 to Val 1256 of SEQ
4 ID NO:2, or wherein the protein comprises a sequence at least 80% identical to the
5 sequence of SEQ ID NO:8 fused to a sequence at least 80% identical to the amino acid
6 sequence inclusive of Lys 677 to Val 1256 of SEQ ID NO:2 via at least one of a chemical
7 or amino acid linking group.

1 56. The protein of claim 51, wherein the HER-2/neu extracellular
2 domain is fused to the HER-2/neu intracellular domain via a chemical linker.

1 57. The protein of claim 56, wherein the chemical linker is an amino
2 acid linker.

1 58. A nucleic acid molecule encoding the protein of claim 51.

1 59. A viral vector comprising a polynucleotide sequence encoding the
2 protein of claim 51.

1 60. A pharmaceutical composition comprising the protein of claim 51,
2 and a pharmaceutically acceptable carrier or diluent.

1 61. The pharmaceutical composition of claim 60, wherein the
2 pharmaceutical composition is a vaccine.

1 62. The pharmaceutical composition of claim 60, further comprising an
2 immunostimulatory substance.

1 63. The pharmaceutical composition of claim 62, wherein the protein is
2 presented in an oil-in-water emulsion.

1 64. The pharmaceutical composition of claim 62, wherein the
2 immunostimulatory substance is SBAS2, 3D-MPL, QS21, or a combination of 3D-MPL
3 and QS21.

1 65. A pharmaceutical composition comprising the nucleic acid
2 molecule of claim 58, and a pharmaceutically acceptable carrier or diluent.

1 66. The pharmaceutical composition of claim 65, wherein the
2 pharmaceutical composition is a vaccine.

1 67. The pharmaceutical composition of claim 65, further comprising an
2 immunostimulatory substance.

1 68. The pharmaceutical composition of claim 65, wherein the nucleic
2 acid molecule is a DNA molecule.

1 69. A method for eliciting or enhancing an immune response to HER-
2 2/neu protein, the method comprising the step of administering to a warm-blooded animal
3 the protein of claim 51 in an amount effective to elicit or enhance the immune response.

1 70. The method of claim 69, wherein the protein is administered in the
2 form of a vaccine.

1 71. A method for eliciting or enhancing an immune response to HER-
2 2/neu protein, the method comprising the step of administering to a warm-blooded animal
3 the nucleic acid molecule of claim 58 in an amount effective to elicit or enhance the
4 immune response.

1 72. The method of claim 71, wherein the nucleic acid molecule is in
2 the form of a vaccine.

1 73. The method of claim 71, wherein the step of administering
2 comprises transfecting cells of the warm-blooded animal *ex vivo* with the nucleic acid
3 molecule and subsequently delivering the transfected cells to the warm-blooded animal.

1 74. A method for eliciting or enhancing an immune response to HER-
2 2/neu protein, the method comprising the step of administering to a warm-blooded animal
3 the viral vector of claim 59 in an amount effective to elicit or enhance the immune
4 response.

1 75. The method of claim 74, wherein the step of administering
2 comprises infecting cells of the warm-blooded animal *ex vivo* with the viral vector and
3 subsequently delivering the infected cells to the warm-blooded animal.

1 76. A method for inhibiting the development of a cancer in a patient,
2 the method comprising the step of administering to a patient an effective amount of a
3 fusion polypeptide according to claim 1, 26, or 51 and thereby inhibiting the development
4 of a cancer in the patient.

1 77. A method for inhibiting the development of a cancer in a patient,
2 the method comprising the step of administering to a patient an effective amount of a
3 polynucleotide according to claim 8, 33, or 58 and thereby inhibiting the development of
4 a cancer in the patient.

1 78. A method for inhibiting the development of a cancer in a patient,
2 the method comprising the step of administering to a patient an effective amount of an
3 antigen-presenting cell that expresses a fusion polypeptide according to claim 1, 26, or
4 51, and thereby inhibiting the development of a cancer in the patient.

1 79. A method according to claim 78, wherein the antigen-presenting
2 cell is a dendritic cell.

1 80. A method according to any one of claims 76-79, wherein the
2 cancer is breast, ovarian, colon, lung or prostate cancer.

1 81. A method for removing tumor cells from a biological sample, the
2 method comprising the step of contacting a biological sample with T cells that
3 specifically react with a HER-2/neu fusion protein, wherein the fusion protein comprises
4 an amino acid sequence that is encoded by a polynucleotide sequence selected from the
5 group consisting of:

6 (i) polynucleotides recited in any one of ^{claims} ~~SEQ ID NO.~~ 8, 33, or
7 58; and

8 (ii) complements of the foregoing polynucleotides;
9 wherein the step of contacting is performed under conditions and for a
10 time sufficient to permit the removal of cells expressing the antigen from the sample.

1 82. A method according to claim 81, wherein the biological sample is
2 blood or a fraction thereof.

1 83. A method for inhibiting the development of a cancer in a patient,
2 comprising the step of administering to a patient a biological sample treated according to
3 the method of claim 81.

1 84. A method for stimulating and/or expanding T cells specific for a
2 HER-2/neu fusion protein, the method comprising the step of contacting T cells with one
3 or more of:

- 4 (i) a fusion protein according to claims 1, 26, or 51;
5 (ii) a polynucleotide encoding such a fusion protein; or
6 (iii) an antigen presenting cell that expresses such a fusion protein;
7 under conditions and for a time sufficient to permit the stimulation and/or
8 expansion of T cells.

1 85. An isolated T cell population, comprising T cells prepared
2 according to the method of claim 84.

1 86. A method for inhibiting the development of a cancer in a patient,
2 the method comprising the step of administering to a patient an effective amount of a T
3 cell population according to claim 85.

1 87. A method for inhibiting the development of a cancer in a patient,
2 the method comprising the steps of:

3 (a) incubating CD4⁺ and/or CD8⁺ T cells isolated from a patient with
4 at least one component selected from the group consisting of:

- 5 (i) a fusion protein according to claims 1, 26, or 51;
6 (ii) a polynucleotide encoding such a fusion protein; and
7 (iii) an antigen-presenting cell that expresses such a fusion
8 protein;

9 such that T cells proliferate; and

10 (b) administering to the patient an effective amount of the proliferated
11 T cells, thereby inhibiting the development of a cancer in the patient.

1 ~~88.~~ A method for inhibiting the development of a cancer in a patient,
2 the method comprising the steps of:
3 (a) incubating CD4⁺ and/or CD8⁺ T cells isolated from a patient with
4 at least one component selected from the group consisting of:
5 (i) a fusion protein according to claims 1, 26, or 51;
6 (ii) a polynucleotide encoding such a fusion protein; and
7 (iii) an antigen-presenting cell that expresses such a fusion
8 protein;
9 such that T cells proliferate;
10 (b) cloning at least one proliferated cell; and
11 (c) administering to the patient an effective amount of the cloned T
12 cells, thereby inhibiting the development of a cancer in the patient.

1 89. A method of making a fusion protein according to claims 1, 26, or
2 51, the method comprising the steps of:
3 (a) introducing into a cell an expression vector comprising a
4 polynucleotide according to claims 8, 33, or 58;
5 (b) culturing the transfected cell; and
6 (c) purifying the expressed protein.

1 90. The method of claim 89, wherein the cell is a CHO cell.

1 91. The method of claim 89, wherein the cell is cultured in suspension,
2 under serum-free conditions.

1 92. The method of claim 89, wherein the expressed protein is purified
2 by a two-step procedure, the procedure comprising:
3 (a) anion exchange chromatography on Q sepharose High Performance
4 Columns; and
5 (b) hydrophobic chromatography on Phenyl Sepharose 6 Fast Flow
6 low substitution.

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